Microgravity: A Novel Environment for Cells

Physical Forces in Evolution

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Physical factors that influence nature

- As life evolved on earth a multiplicity of physical factors participated in the complicated selection process. For many factors there are clear examples of the role of physical forces in determining the pathways in evolution. A notable exception is gravity. The force of gravity has been relatively constant for the duration of the evolutionary processes on Earth. Therefore as we transition terrestrial life to low gravity environments and study the adaptive processes in cell, our understanding of the role of gravity in shaping evolution on Earth will increase. Additionally investigations in lower species with short generation times may reveal suites of characteristics favor competition, survival, and eventual thriving in low gravity.

Physical factors that influence nature

- **Thermal**
  - Thermophiles
  - Psychrophiles
- **Hydrodynamic**
  - Shear
  - Hydrostatic pressure
- **Convection**
  - Gravity
  - Surface tension driven (Marangoni)
- **Mechanical**
  - Impact
  - Vibration
- **Microgravity**
  - Direct
  - Indirect
- **Ionizing Radiation**
  - Ultraviolet
  - Gamma and cosmic

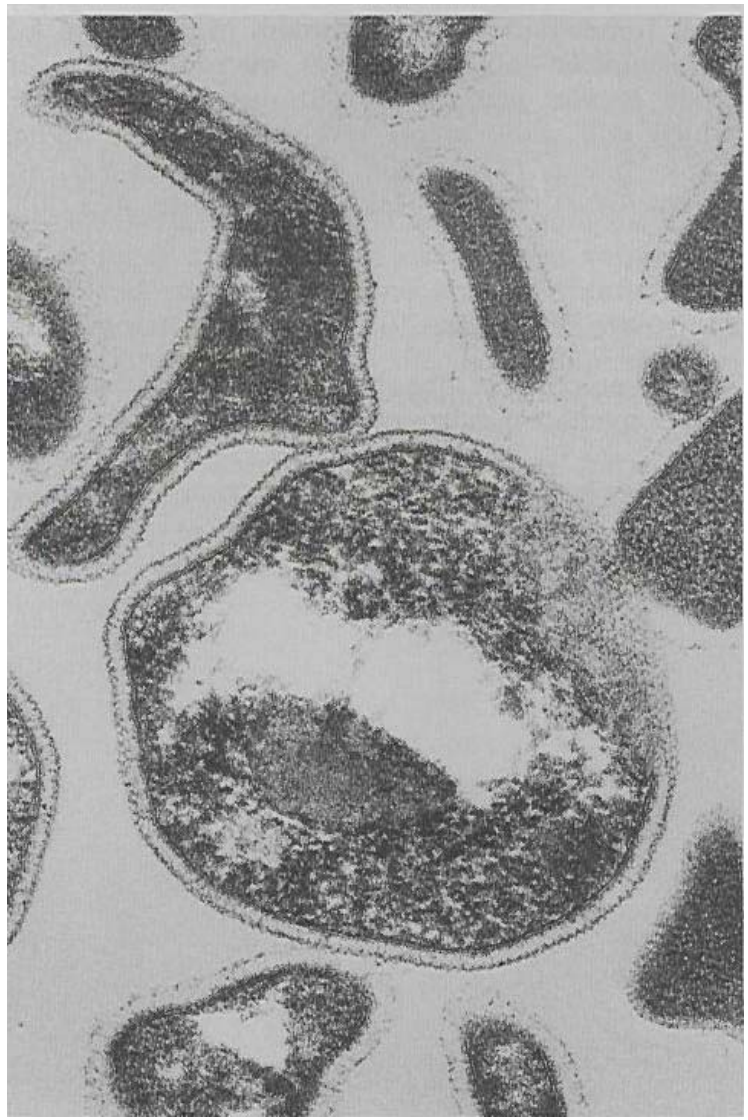
Microgravity

- Indirect
  - Loss of gravity driven convection
  - No sedimentation
  - Diffusion limitation of access to nutrients
  - Diffusion limitation of waste dissipation
- Direct
  - Shape change

Thermophiles

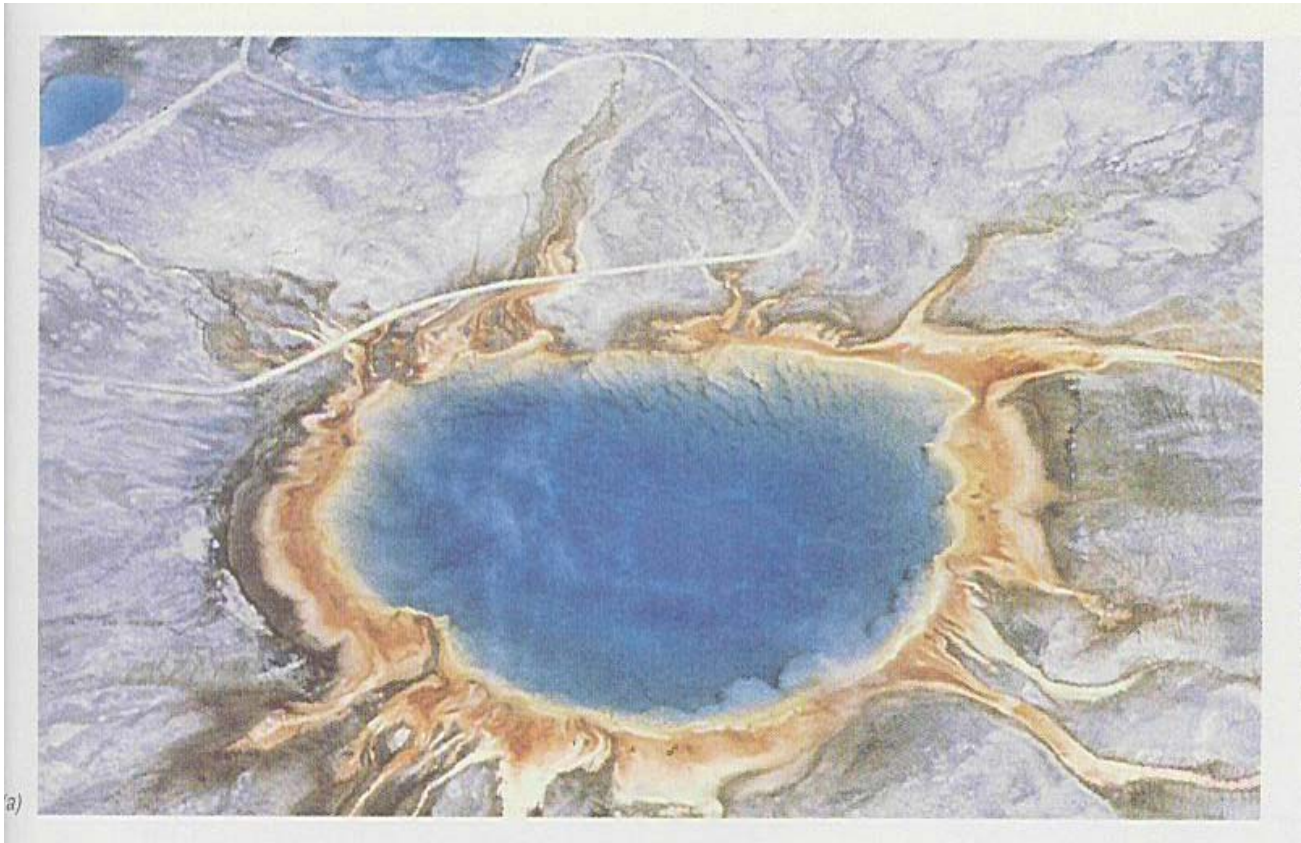
Found in hot springs, ocean thermal vents, aqueous and gaseous thermal pollution, and adjacent to active volcano sites.

Cells and organisms adapted to more temperate environs respond to temperature elevation with changes in gene expression followed by synthesis of the heat shock proteins.



Pyrodictium sp.
Optimum temp is 105^{o}C

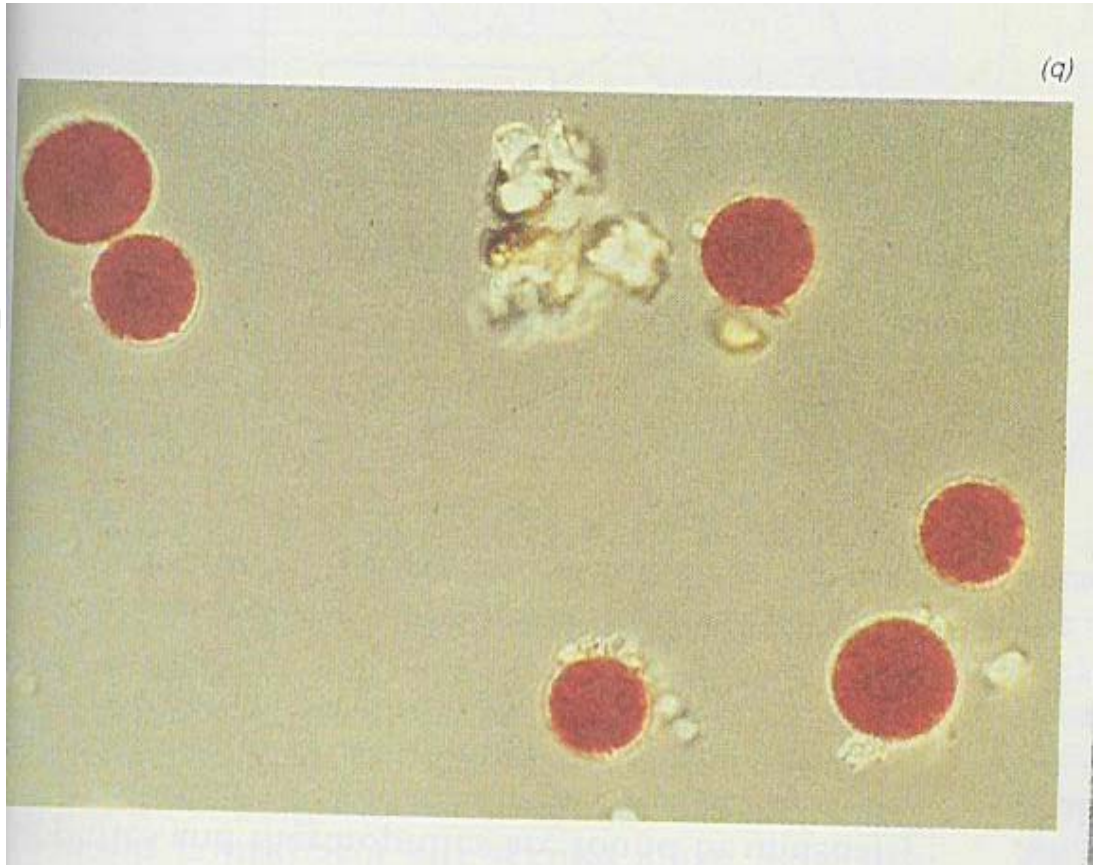
Thermophiles



Cyanobacteria in a hot spring. Orange color is carotenoid pigment from the organism. Optimum Temperature is 70^{o}C.

Psychrophiles

Propagate optimally at 15°C or lower, even below freezing. Replication rate precipitously declines above 20°C



Snow algae (Chlamydomonas)

Three Abiotic Factors and Selected Microorganisms that Grow Under Extreme Conditions in Nature

Abiotic Factor	Microorganism[d]	Conditions for Growth
Temperature	***Pyrodictium***	82° C and above
	Thermoactinomyces spp.	68° C
	Rhodotorula sp.[a]	14° C
	Flavobacterium spp.	4° C
	Bacillus globisporus	-10° C
pH	*Agrobacterium sp.*	12.0
	Vibrio cholerae	9.0
	Bacillus pasteurii	8.5
	Sulfolobus sp.	0.5
	Thiobacillus thiooxidans	0
Osmotic pressure	*Candida spp.*[a]	60% sugar[b]
	Hansenula spp.[a]	60% sugar[b]
	Saccharomyces spp.[a]	60% sugar[b]
	Halobacterium salinarum	27-30% NaCl[c]
	Sarcina morrhuae	27-30% NaCl[c]
	Pseudomonas cepacia	Distilled water

[a] These organisms are yeasts; all others are bacteria.
[b] Honey is one example of such a high sugar concentration in nature.
[c] This concentration of NaCl can be found in Great Salt Lake, Utah, and the Dead Sea.
[d] For pronunciations, see the Organisms Pronunciation Guide at the end of the text.

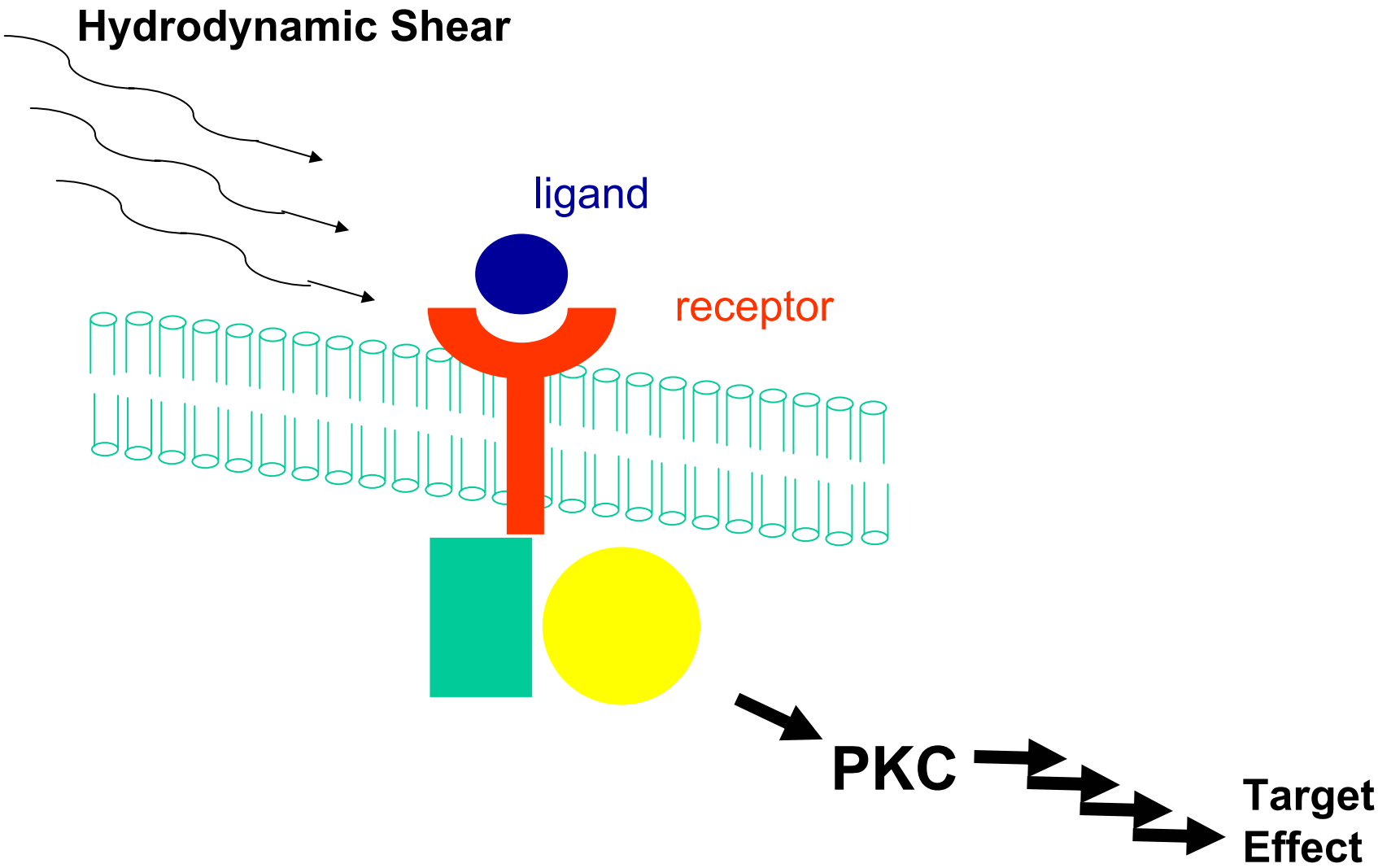
Hydrodynamic shear

- Force generated by fluid moving past a fixed object or an object moving at a greater or lesser rate or in a direction opposing the flow.
- Examples
  - Estuarial and aerial organisms
  - Cells in the vascular compartment
    - RBC's and WBC's
    - Endothelial cells

Hydrodynamic shear

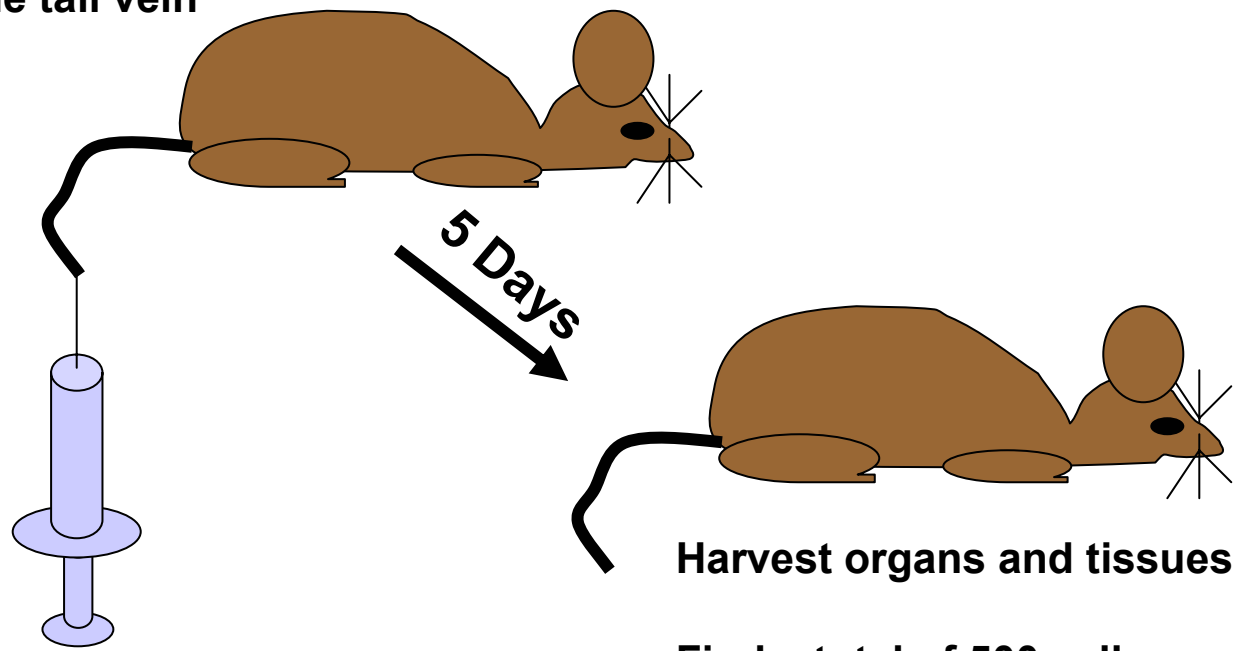
- Effects (Negative)
  - Death
  - Changes in membrane composition
  - Altered signal transduction
- Effects (Positive)
  - Mass transfer
  - Differentiation
  - Facilitates renewal

Physical Principles in Space Biology



Hydrodynamic shear

Inject 100,000 syngeneic melanoma tumor cells iv into the tail vein

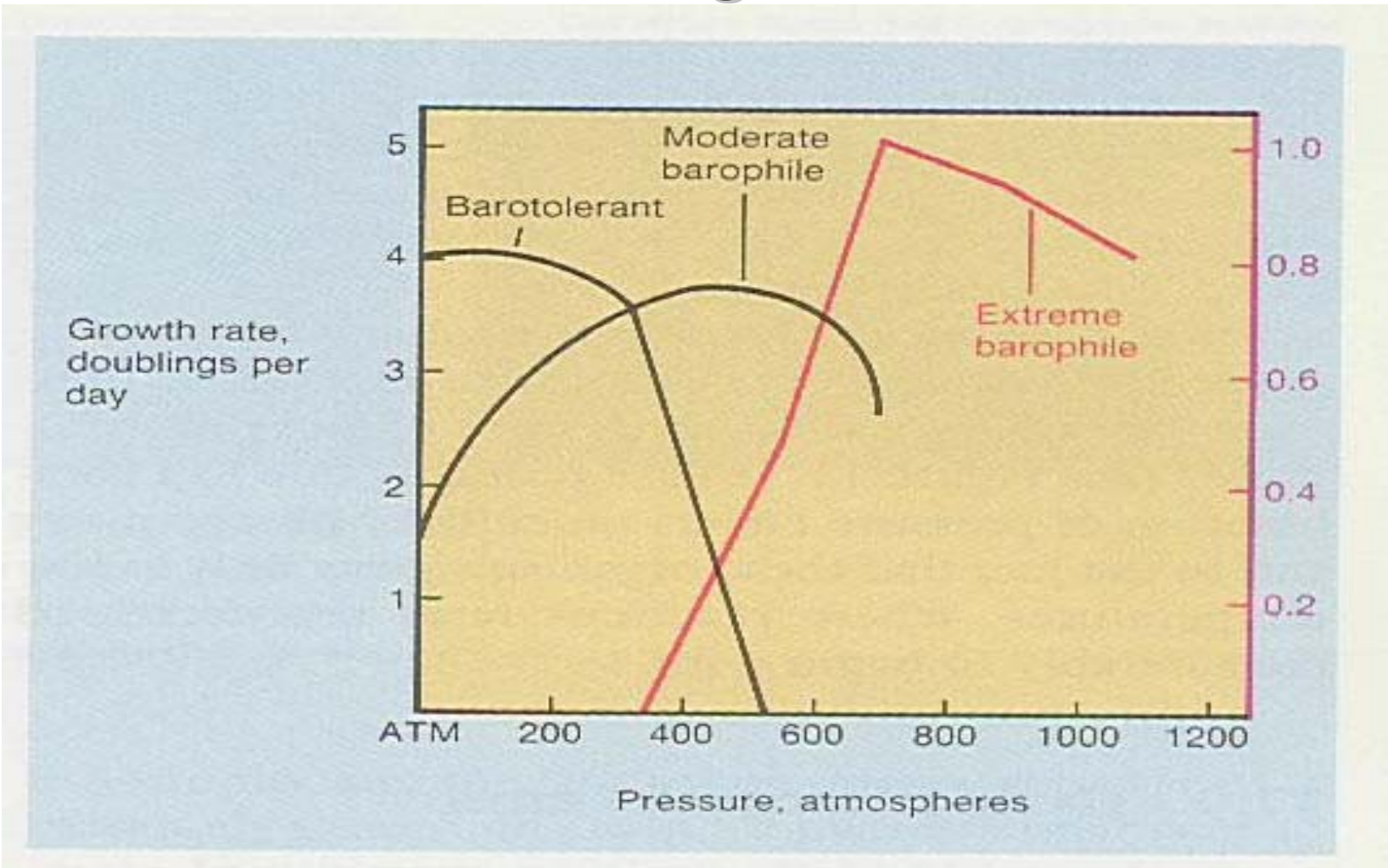


Harvest organs and tissues

Find a total of 500 cells. 300 are in the lungs. Where are the other 99,500 cells??

Hydrostatic Pressure

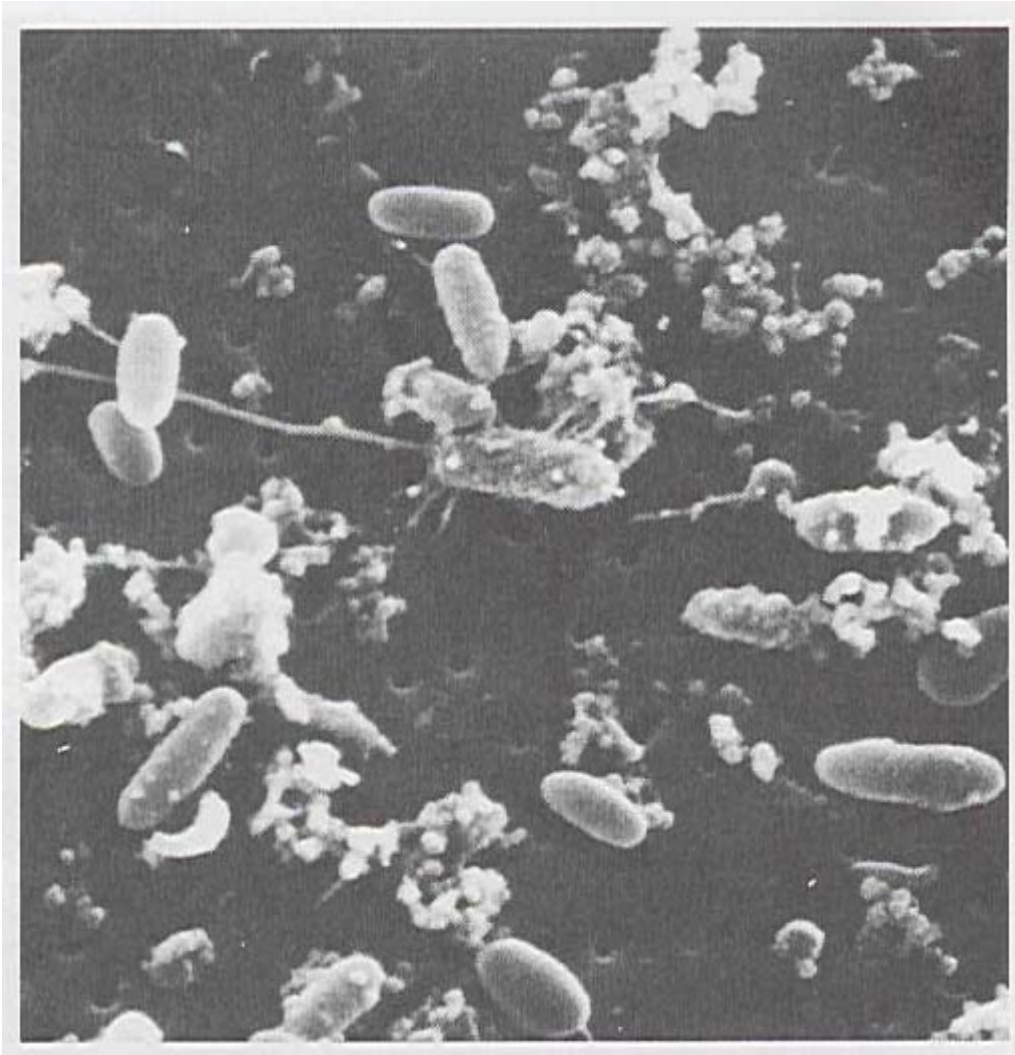
Micro-Organisms



Barophiles

Piezophiles

May require as much as 700 atm in order to grow. Found in deep ocean trenches.

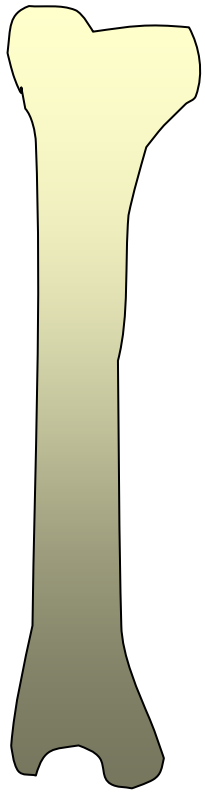


Hydrostatic Pressure

Various cells within the body may respond to and rely upon hydrostatic pressure gradients for normal function.

Bone development, maintenance, and renewal may depend on physical force profiles that include hydrostatic pressure along with the load of the body.

Frangos et al. 2001



Convection

- Movement within a fluid based on intrinsic or induced differential density
- Gravity dependent phenomenon
  - Partitioning of oil and vinegar
  - AC systems
  - Facilitation of mass transfer
- Surface tension driven convention
  - Significant in the absence of gravity

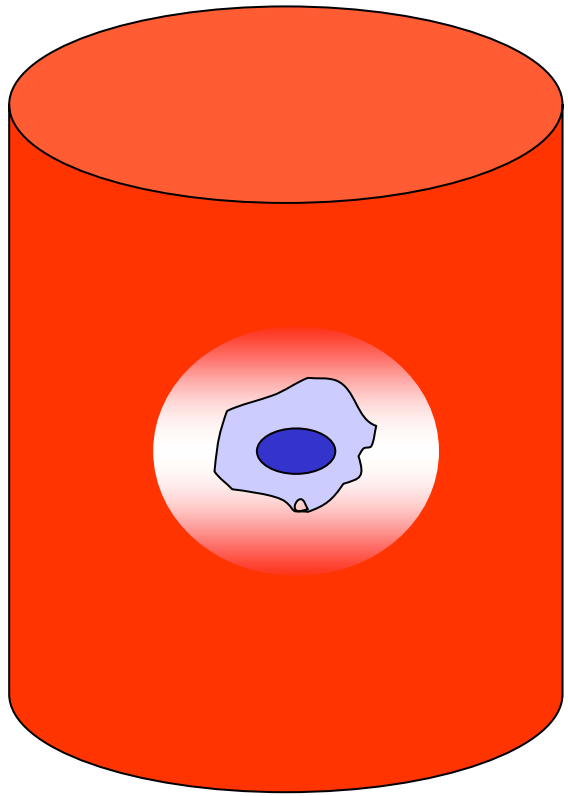
Mechanical

- Impact
  - Seldom in nature (cells)
  - Artificially in cell culture (spinner culture)
- Vibration
  - Selective role unknown
  - Repetitive use injury
  - A confounder in determining the role microgravity in cellular responses
  - Early results suggest that different cell populations have different frequency optima
  - First responses frequently involve transcription factors

Microgravity

Indirect

- Absence of gravity driven convection
- Nutrient transfer limited to the rate of diffusion
- Cells become anoxic

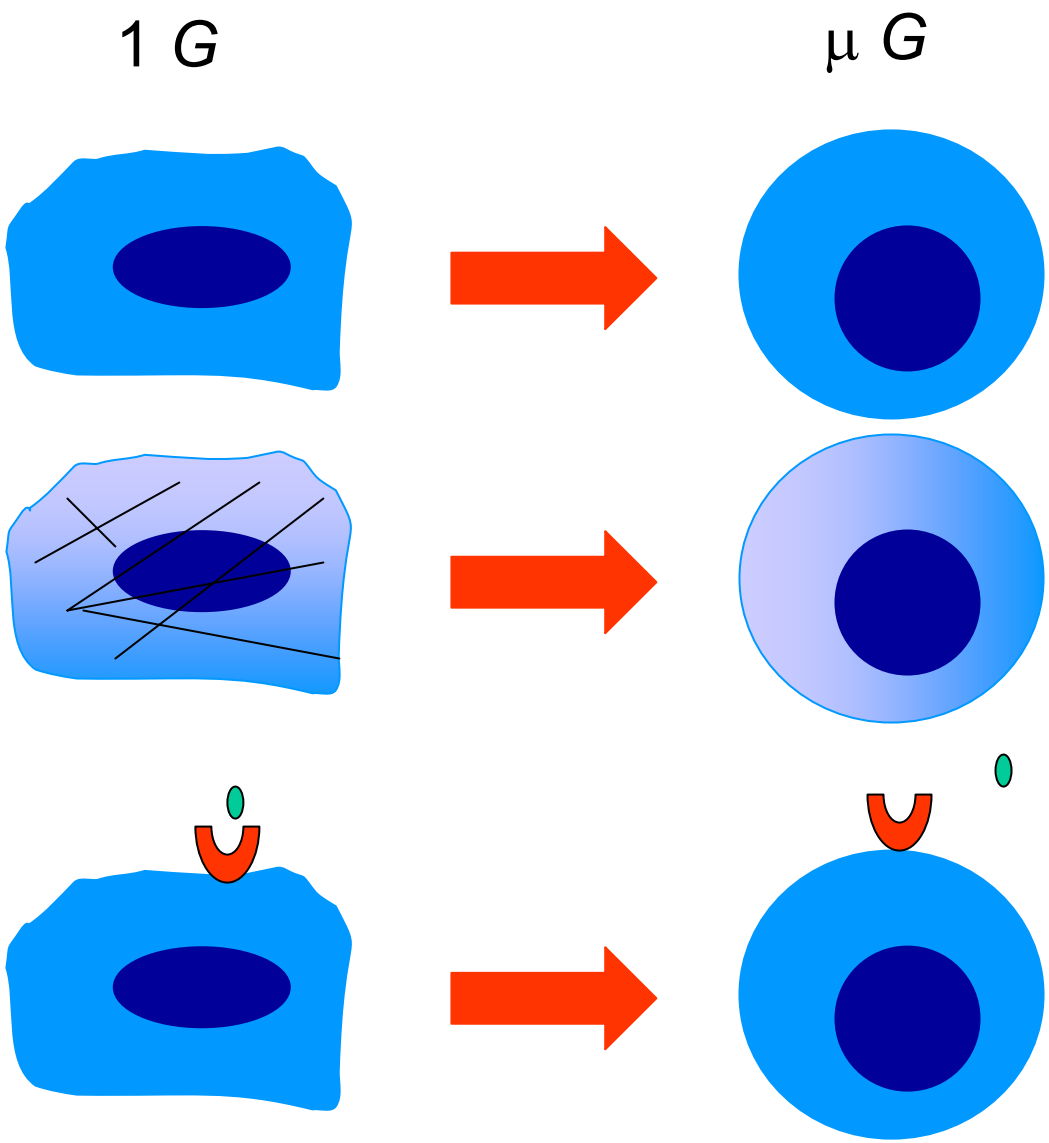


Microgravity

Direct

- No surface attachment
- Cells tend toward spherical shape unless previously attached to a surface
- Disorganization of MTOC's (microtubule organizing centers)
- Membrane lipid raft changed
- Transmembrane signalling for some receptor mediated activities
- Induction of differentiation
- Delay in onset or inhibition of apoptosis
- Inhibitition of locomotory activity
- Potential exaggeration of cell-cell rather than cell-substratum interaction

Physical Principles in Space Biology



- Shape change
- Locomotion
- $P_{in} \sim P_{out}$

- **Viscosity gradient change**
- **Redeployment of cytoskeleton**
- **Fluid movement**
- **Gene expression**

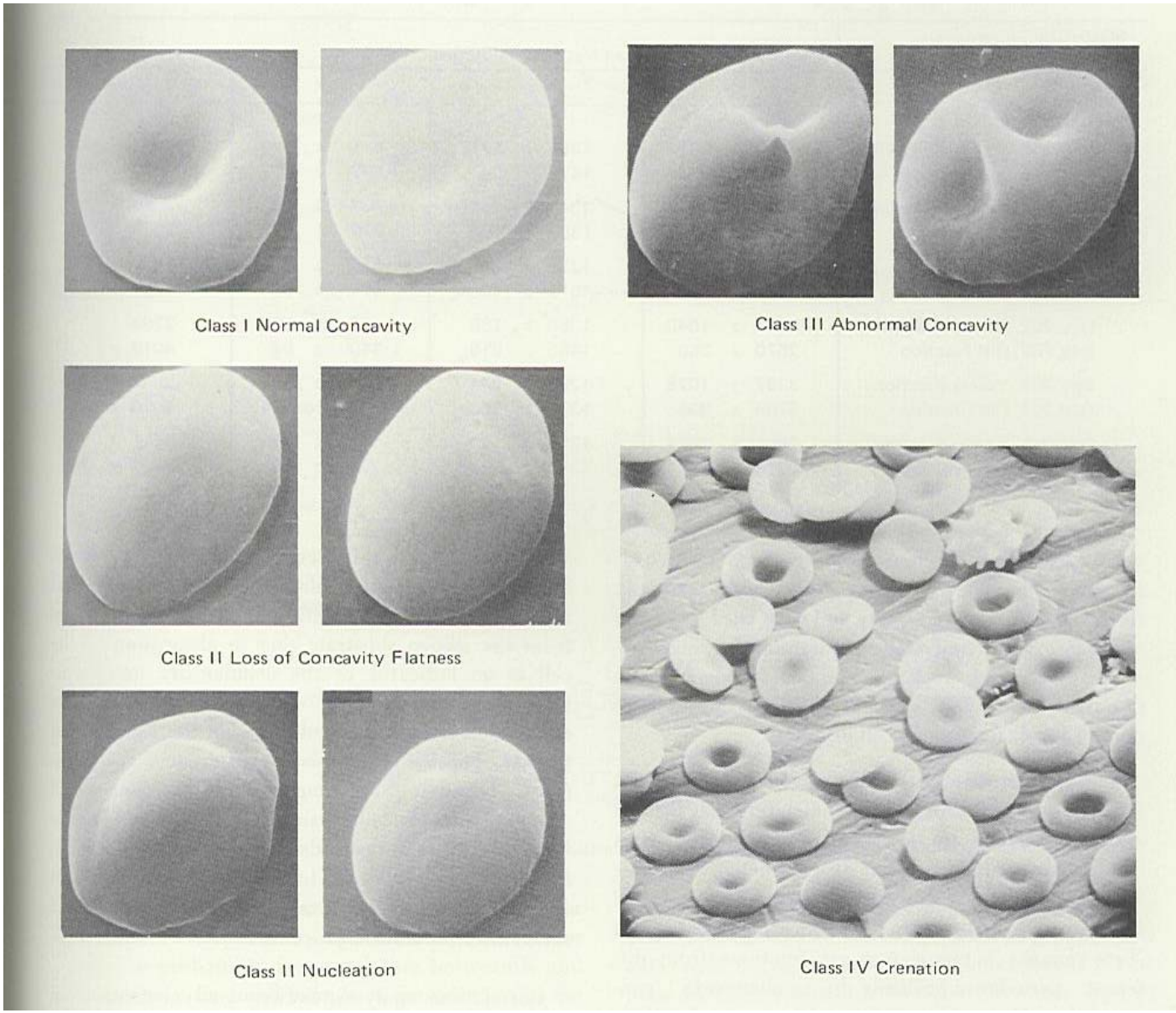
- Signal transduction
- Apoptosis
- Receptor-ligand interaction
- Internal signal pathways

Red Blood Cell Abnormalities

Skylab Results

SEM of blood drawn on orbit.

Controls were Preflight and Chamber crew



Growth of WI-38 Cells in Microgravity

No significant difference

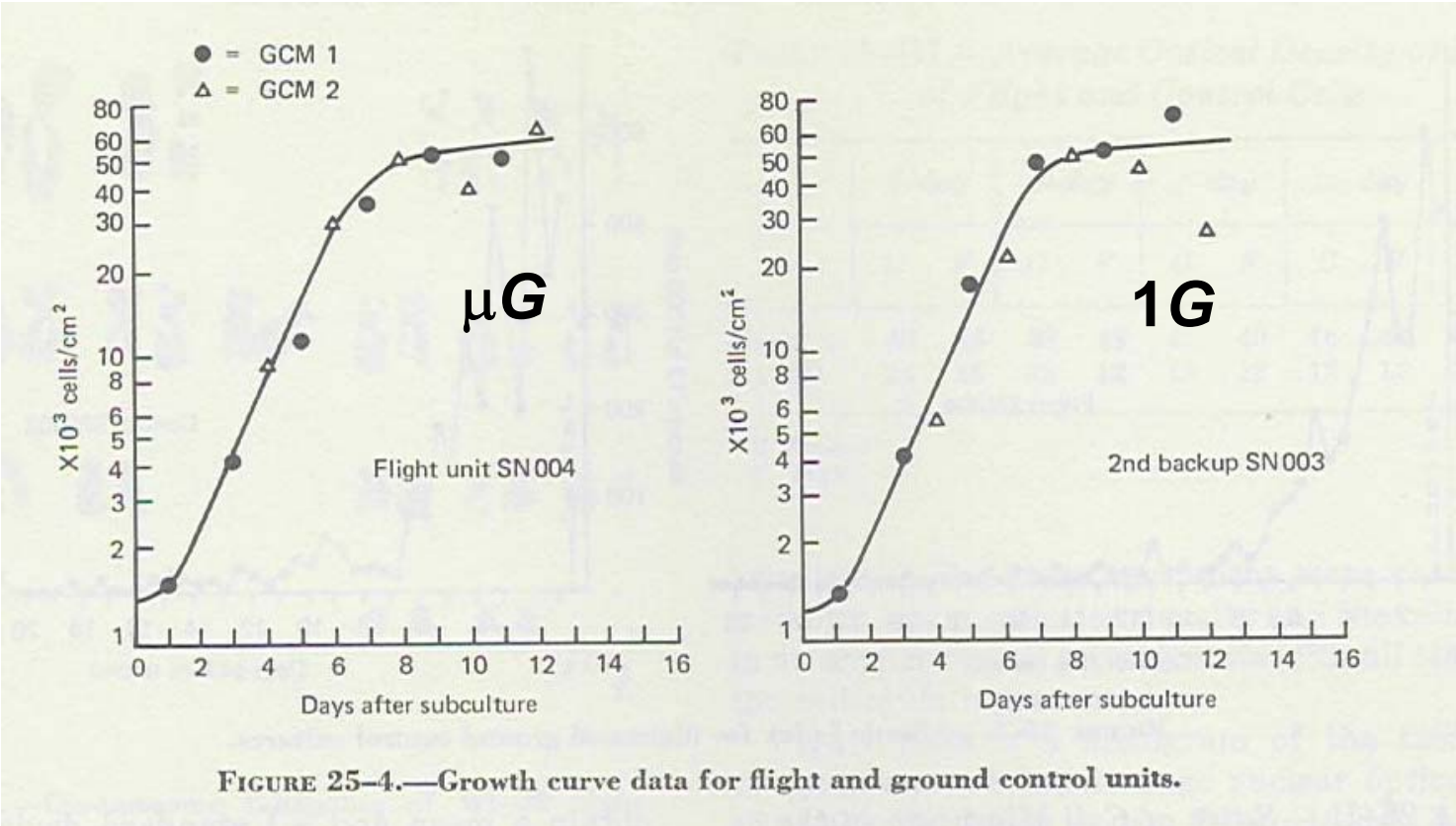


FIGURE 25–4.—Growth curve data for flight and ground control units.

Montgomery et al. In 'Biomedical results from Skylab' pp 221-234 (1977) Science and Technical Office, NASA

Growth of WI-38 Cells in Microgravity

Human embryonic lung cells

Note the overlapping

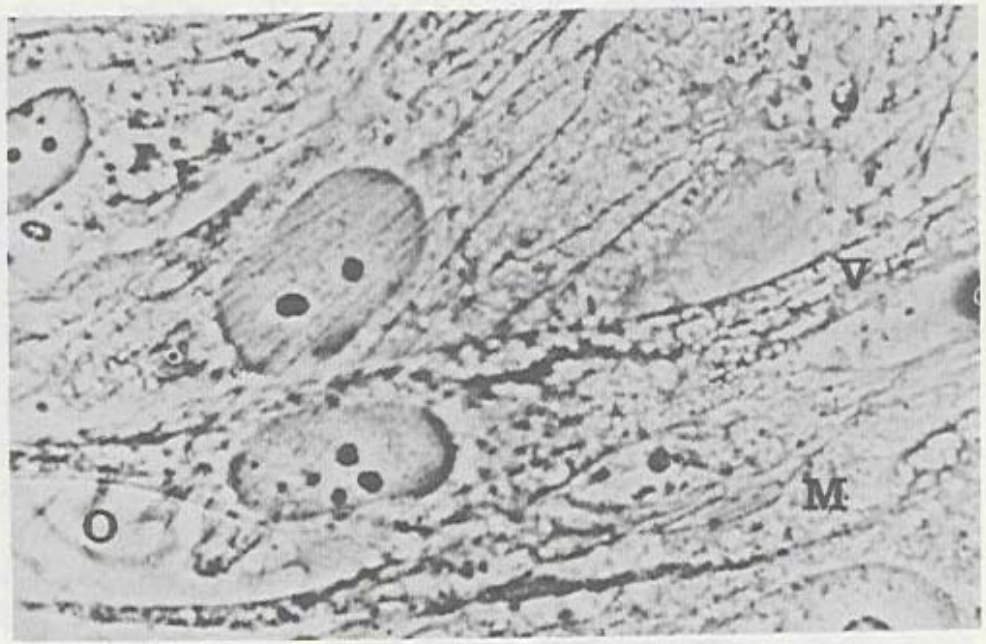
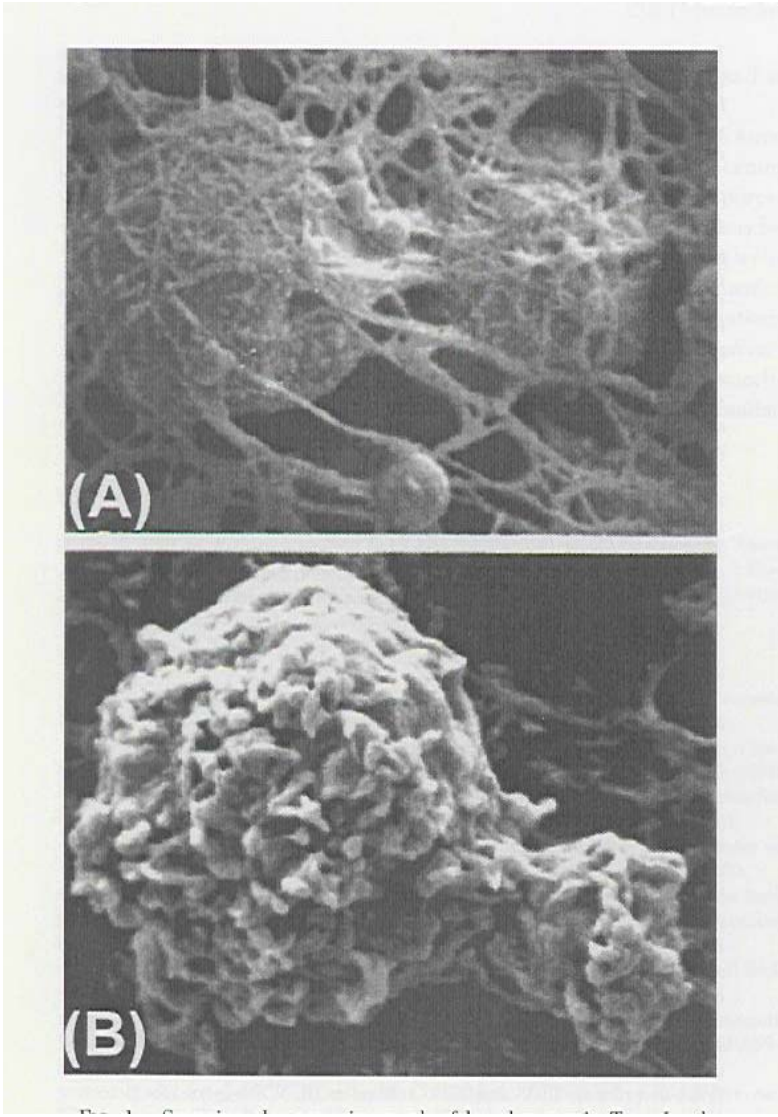


FIGURE 25–10.—Phase photomicrograph of 8-day-old WI–38 cell culture. Note overgrowth of one cell over another producing a multilayered colony (O). Numerous clear vacuoles are present (V). A few filamentous mitochondria (M) are present. Glutaraldehyde and osmium tetroxide fixation. Gelatin-phenol mount. Magnification 2000 ×.

Human Lymphocytes

Microgravity and Analog Culture--- The morphologic Characteristics are the same; The cells are spherical.

In 1*G* cells polarize by extending Podia as a prelude to locomotion



Summary

- Indeed microgravity affords a unique environment for cells
- The response of cells to microgravity and space must undergo a careful dissection to understand the direct contribution of microgravity
- Thus, we must facilitate mass transfer and account for the shear, vibration, and other physical invoked by cell culture in space.

Assignment

Tensegrity Model

Physical forces, such as those due to gravity, play an important role in tissue development and remodeling. Yet, little is known about how individual cells sense mechanical signals or how they transduce them into a chemical response. Rather than listing the numerous signal pathways that have been found to be sensitive to mechanical stimulation, we need to place potential molecular signaling mechanisms within the context of the entire cell. The model presented is based on the concept that cells use tensegrity architecture to organize their cytoskeleton and stabilize their form. Studies with stick and string tensegrity cell models predict that living cells are hard-wired to respond immediately to external mechanical stresses. This hard-wiring exists in the form of discrete cytoskeletal filament networks that mechanically couple specific cell surface receptors, such as integrins, to nuclear matrix scaffolds and to potential transducing molecules that physically associate with the cytoskeleton. If these signaling molecules do function in a "solid-state", then mechanical stresses may be transduced into biochemical responses through force-dependent changes in cytoskeletal geometry or through local alterations in thermodynamic or kinetic parameters. Changes in cytoskeletal tension (prestress) also may play a role in signal amplification and adaptation.

Ingber, D. Gravit Space Biol Bull Jun;10(2):49-55 (1997)

Annoying Question No. 1

- How would you determine in cell culture if there are cooperative effects from two or more physical stressors in 1*G* and in μ*G*?